

IMPACT OF ENZYMATIC PROTEIN CROSS-LINKING ON GELATION KINETICS AND MICROSTRUCTURE OF RENNET-INDUCED LOW-FAT MILK GEL

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ABSTRACT

A high potential for modifying the texture properties of casein based dairy products through protein cross-linking by microbial transglutaminase (TG) has recently attracted considerable attention. Hereby, the impacts of different levels of TG and heat treatment of milk on the rennet gelation kinetics and microstructure properties of low-fat milk gel (~70% fat reduction) were investigated. The rheological analysis was measured by a dynamic rheometer and by a formagraph. In order to obtain complementary insights into the protein network formed, the gel microstructure was measured using confocal laser scanning microscopy. The results show that the influence of TG on the rennet coagulation properties appears more intense in the secondary phase measurable by; marked increase in curd yield percentages, intensive reduction in curd firmness, declined rigidity rate of casein network and markedly reduction in gel strength, than in the rennet primary phase represented in prolongation of the coagulation times. A denser and more homogeneous systematic protein aggregate network accompanied by finer, smaller and evenly distributed pores, were observed in TG-treated gels. These effects on gelation kinetics and microstructure were more pronounced for higher TG concentrations and higher heat treatment of milk. A highly significant ($P < 0.001$) and strong correlations were obtained among all formagraph and rheometer parameters. In sum, TG cross-linking intensely altered the functional properties of the resulting low-fat milk gel that might allow manifold applications and enhancement of low-fat cheese quality attributes.

Keywords: Transglutaminase, gelation kinetics, microstructure, rheometer, formagraph.

INTRODUCTION

Milk gels have both traditional and high commercial value within the dairy industry. The overall visual appearance, microstructure and rheology are important attributes. These attributes contribute to the overall sensory perception and functionality of milk gels, and consequently their respective based dairy products.

Even though the concept of low-fat dairy product manufacture is dated from the early 50s, the consensus on control of caloric intake, especially in developed countries, has largely been responsible for the growth of the low-fat dairy products markets resulting in accelerated commercialization of low-fat dairy products in the past two decades (Mistry, 2001). However, consumers often regard dairy products group of low- or reduced-fat to have inferior quality. Nevertheless, the novel technology in

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dairy industry and the significant advances in understanding the biochemical and physicochemical characteristics of low-fat variants have led to a potential improvement in terms of flavour, texture and functionality (Sharma *et al.*, 2001).

Enzymatic cross-linking is one of the several attempts that have been carried out to overcome the problems and quality defects linked to the reduced-fat dairy products during the last decade (Bönisch *et al.*, 2008). Transglutaminase (TG) is an enzyme (EC 2.3.2.13) that capable of forming both inter- and intra-molecular isopeptide bonds in and between protein-bound glutamine and lysine residue with the formation of ϵ -(γ -glutamyl)-lysine linkages (Rodriguez-Nogales, 2006; Bönisch *et al.*, 2008). Traditional milk protein gels are stabilized mainly by weak non-covalent interactions. Introduction of new covalent bonds by cross-linking leads to gels with enhanced structure and functional properties. Hereby, the use of TG offers such opportunity to improve the rheological and other quality properties of dairy products by means of improving elasticity and water-holding capacity (Bönisch *et al.*, 2007). Besides, it can improve the nutritive value of dairy products through cross-linking of milk casein and whey proteins containing complementary essential amino acids (Faergement *et al.*, 1999; Schorsch *et al.*, 2000). Various reviews demonstrate the high potential of modifying the texture properties of casein-based dairy products by means of TG cross-linking (Ozrenk, 2006, De Jong and Koppelman, 2002; Jaros *et al.*, 2010).

Certainly, the functional properties of dairy product ingredients are dedicated by the structure. The protein based dairy products (i.e. cheese, yogurt and fermented milks) are stated to have a microstructure consisting mostly of the casein matrix in which the fat globules are entrapped; water or serum is both bound to casein and fills interstices of the matrix that forms a network (Hort and Grys, 2001). Thus defining the structural properties will increasingly become a critical criterion for dairy manufacturers seeking to design new products, to maintain the quality of current ones or understand the strengths and weaknesses of the new relative to their competitors. In this context, confocal laser scanning microscopy (CSLM) is a technique that has great potential as a tool to improve our understanding of dairy product microstructure. Compared to conventional microscopic techniques, CSLM offers a number of advantages. It can both make visible and chemically differentiate the dairy product components through the use of specific stains. Additionally, CSLM has proven to be very useful technique for examination of highly-hydrated and high-fat foods without the loss or migration of their components.

The objective of the present study is to investigate the impact of the level of TG addition on the coagulation kinetics and the microstructural characteristics of rennet-induced low-fat milk gels (~70% fat reduction). Also, investigate if increasing the intensity of heat treatment could be used to enhance the enzymatic cross-linking and its influence on the properties of the resulting low-fat milk gels. A further aim was to use the General Linear Model (GLM) analysis to explore the correlation between the rheometer and the formagraph assessments of milk gelation, in order to explore the influence of

the rheological methodology on defining the physical properties of the milk gels.

MATERIALS AND METHODS

Fresh whole cow milk was subjected to separation up to ~ 70% fat reduction (giving ~ 1.1% fat). The microbial rennet (CHY-MAX[®] M, Christian Hansen, Milwaukee, WI 53214, USA) was used. The used microbial TG enzyme was Activa[®] YG (E.C. 2.3.2.13) (Ajinomoto Foods Europe S.A.S., Hamburg, Germany). According to the product specification this enzyme preparation is recommended for cross-linking of milk proteins in unheated or pasteurized milk, and it has a declared specific activity of 100 U g⁻¹ powder.

Rennet-induced coagulation was performed in an experimental block design (three replicate blocks) with two experimental factors; Factor 1. Heat treatment of milk (2 levels): 72°C/15sec (T₇₂) or 85°C/5min (T₈₅); Factor 2. Concentration of TG (3 levels): without TG (0U), 1 (1U) and 2 (2U) units TG/g milk protein.

All low-fat milk portions were heat treated according to their proposed temperatures in double-walled stainless-steel vats and then cooled to 35°C. TG concentrations were initially dispersed in a portion of the heat treated low-fat milk from their respective vats with agitation for 2 min or until no lumps were visible. Six different milk gels were resulted from the combination of three levels of TG (0, 1 and 2 U/g milk protein) and two different heat treatments (72°C/15sec and 85°C/5min) as follow: T₇₂-0U (72°C/15sec and no TG addition), T₇₂-1U (72°C/15sec with 1U TG/g milk protein), T₇₂-2U (72°C/15sec with 2U TG/g milk protein), T₈₅-0U (85°C/5min with no TG addition), T₈₅-1U (85°C/5min with 1U TG/g milk protein) and T₈₅-2U (85°C/5min with 2U TG/g milk protein). From the technological viewpoint, TG was added simultaneously with the rennet enzyme as concluded and recommended by Bönisch *et al.* (2008).

Rheological measurements: The gelation kinetics of the viscoelastic milk gels of all treatments were performed by a dynamic rheometer as well as by a formagraph.

The kinetics of milk coagulation at 35°C were monitored in a concentric cylinder geometry (measuring system Z3 DIN of 25mm bob diameter stainless-steel and cup) of a Physica UDS200 Rheometer (Anton Paar Germany GmbH, Ostfildern, Germany) by applying oscillatory strain. After rennet addition; each sample was prepared as described in above, and ~20ml of the prepared milk sample was transferred immediately to the tempered (35°C) rheometer cup. The oscillation measurement started 2 min after rennet addition. The storage modulus (G') that represents the elastic behavior, loss modulus (G'') and loss tangent ($\tan \delta = G''/G'$) were measured at 1 Hz frequency with 0.5 % strain in the linear viscoelasticity range at 35°C for 60 min. The data of the rheological measurements were analyzed with software supporting the rheometer; US200/32 V2.50. In this work, the onset of gelation (t_c) determined at the time when G'' cross G' and $\tan \delta = 1$. According to Volikakis, *et al.* (2004) and Bönisch, *et al.* (2008), the storage modulus G'_{30min} (indicates the rigidity rate of casein network after 30 min

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reaction time) as well as G'_{60min} (defines the gel strength after 60 min reaction time) were determined. Based on this experimental set-up the t_c , G'_{30min} and G'_{60min} can be determined in one single measurement. Each measurement was carried out in triplicate.

The coagulation properties of the milk were measured by a Lattodinamografo (FOSS ITALIA S.p.A., Via Belgio, 35127 Padova, Italy) according to the method of McMahon and Brown (1982). Clotting time (RCT), time required to achieve a curd firmness of 20 mm (K20) and curd firmness measured 30 min after the addition of rennet (A30) were recorded at 35°C. The overall time of every formagraph run was 60 min. Measurements were carried out in at least triplicate in the same milk sample. Finally, Comparison and correlation between rheometer parameters (i.e. the onset of gelation and G'_{30min}) and formagraph parameters (i.e. RCT and A30) were conducted by GLM analysis.

The yield was determined by a centrifugation method, essentially as described by Bönisch *et al.* (2008). 100 g of coagulated milk samples were centrifuged at 3000_g at 20°C for 15 min with a BECKMAN centrifuge (Analytical Instruments LLC, Minneapolis, USA). The released serum was decanted and the sediment was determined gravimetrically. The yield after centrifugation in % (w/w) was defined as the weight of the gel attained after centrifugation (m_{gel}) in relation to the initial weight of milk sample (100g) according to Eq. (1). All measurements were carried out in triplicate.

$$\text{Yield [\%]} = \frac{m_{gel} [\text{g}]}{100 [\text{g}]} \times 100 \quad (1)$$

Confocal Laser Scanning Microscopy (CLSM): From the fresh prepared milk gels, small cylindrical pieces from the center of milk gel blocks (approximately 3mm in diameter and 7 mm in height) were prepared and fixed overnight in 4% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 6.8. Thin slices of ~ 2 mm in diameter and height were prepared from the fixed cylindrical samples using a fine scalpel. The protein network was stained using 0.2% (w/v) Fast Green FCF fluorescent dye (Sigma-Aldrich, UK), where 0.01% Nile Red fluorescent dye (Sigma-Aldrich, USA) was used to label fat. Milk-gel slices were incubated with a mix of both stains for 10 min at ambient temperature. Each slice was placed between a microscope slide and a cover slip. Samples were then examined at 25 °C with a 63x oil objective lens and sequential scan using a Leica TCS NT confocal laser scanning microscope (Leica Microsystems, Heidelberg, Germany), which employed an argon/Krypton laser in dual-beam fluorescent mode, with excitation wavelength of 568 nm for fat and 488 nm for proteins. In the CLSM micrographs, the fat was labeled in green and the protein was labeled in red where the aqueous phase appears as black areas.

The total nitrogen content (TN %) was measured by the Kjeldahl method (International Dairy Federation (IDF), 1993). Total protein content was calculated by multiplying the TN % by 6.38. Milk fat content was determined by the Gerber method (Ling, 1963). Milk total solids (TS %) content was determined according to (AOAC, 1990). pH was measured in the

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fresh milk samples using a digital pH-meter PHM92 (MeterLab™, Radiometer Analytical S.A., France). All samples were analyzed in triplicate.

Analysis of variance (ANOVA) was carried out using a general linear model (GLM) procedure of the software package Minitab® 16 (MINITAB Inc., State College, PA, USA) and significant differences ($P \leq 0.01$) between treatments were determined.

RESULTS AND DISCUSSION

The average composition and pH values of fresh cow milk used in the present study are given in Table 1. Reduction of the fat content (~ 70 %) of milk reduced its total solids (TS %). Whereas, the total protein content of low-fat milk (the vital factor in the calculation of TG powder addition) was slightly increased. Furthermore, there was no obvious effect on the pH values as a function of milk fat content.

In order to investigate a possible coherency between the serum binding of the gel and the altered gel properties as a function of heat treatment and/or TG concentration, the yield was determined after centrifugation. The curd yield percentages after centrifugation are represented in Fig. 1 and given in Table 2. A significant increase ($P < 0.01$) in curd yield was observed in low fat-milk gels treated with higher heat treatment as the T₈₅ treatments showed a tremendous increase in curd yield varied from 6.2 up to 10.4 % compared to those of T₇₂ treatments (Table 2). Singh and Waungana (2001) stated that the severe heat treatment of milk provides a potential route for maximizing cheese yield by the inclusion of whey proteins in curd as well as changes occurred in casein micelles; i.e. size, hydration of micelles and association reactions. Furthermore, it has been reported that the milk gels of excessive thermal treatment possessed a higher water holding capacity that not only attributed to the incorporation of denaturated whey proteins into the casein network, but further to the remained native whey proteins trapped in the rearrangement of casein micellar structure (Lucey *et al.*, 2001; Li *et al.*, 2005; Rodriguez-Nogales, 2006).

Table (1). Gross composition and pH values of milk used in the manufacture of milk-gels.

	Full-fat milk	Low-fat milk
Fat %	3.69 ± 0.14	1.11 ± 0.05
Protein %	3.39 ± 0.05	3.48 ± 0.07
Total solids %	12.33 ± 0.24	9.95 ± 0.11
pH	6.66 ± 0.03	6.64 ± 0.01

Values are means ± standard deviation.

Regarding the impact of TG addition, it is obvious that curd yield percentage was significantly increased ($P < 0.01$) in low-fat milk gels treated with TG within both heat treatment, compared to their respective control (T₇₂-0U and T₈₅-0U). The data given in Table 2 and depicted in Figure 1 clearly show that the curd yield percentage significantly increased ($P < 0.01$) as the

TG concentration increased. It is worthwhile to note that a marked increase in curd yield percentage (6.02% and 12.7%) was obtained in low-fat milk gels of T₈₅ treatment as a function of increasing TG concentration, compared to those of T₇₂ treatments (5.2% and 8.48%), with respect to their control counterparts without TG addition. This finding is in agreement with that of Bönisch *et al.* (2008) who concluded that the curd yield after centrifugation was increased as a function of increasing TG concentration. Schorsch *et al.* (2000) have also found less syneresis in cross-linked milk gels compared to that of no TG treated. Additionally, Cozzolino *et al.* (2003) indicated a high cheese yield by using TG and had attributed that finding to the incorporation of whey proteins into the cheese curd. .

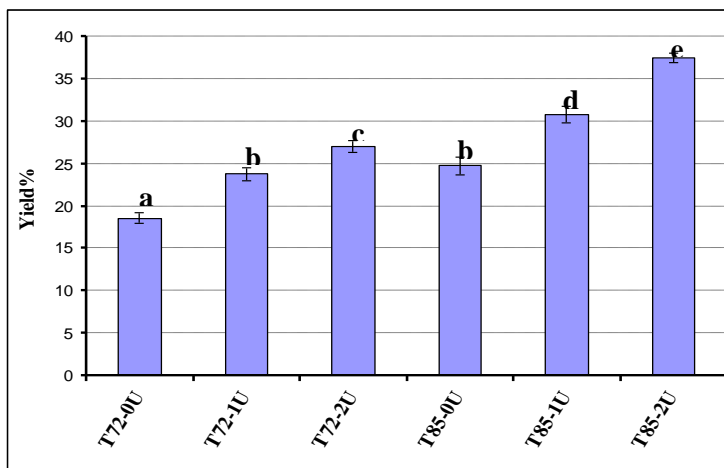


Fig. (1): Curd yield% of low-fat milk gels representing heat treatment and TG concentration effects. Values are means for three different replicates with their standard deviations represented by vertical bars. Different letters denote significant differences ($P < 0.01$).

In this context, Rodriguez-Nogales (2006) stated that milk proteins represent a favorable substrate for transglutaminase enzyme and significant improvements in the enzymatic cross-linking were observed when high heat treatment was applied to milk, and he has correlate that to the denaturalization of whey proteins and the rearrangement of casein micelles during the heat treatment which in turn improve the reactivity of protein substrate towards protein cross-linking. Furthermore, Bönisch *et al.* (2008) elucidated that the increased curd yield is rather explained by the enhanced serum binding of the gel network stabilized by additional covalent bonds than by the incorporation of native whey protein into the gel network.

By the formagraph and the dynamic rheometer techniques, the kinetics of milk coagulation can be monitored and detected. Table (2) shows the milk-clotting parameters derived from formagraph and rheometer at 35°C. Rennet Clotting time (RCT) and the onset of gelation (t_c) values have increased significantly ($P < 0.01$) as a result of adding TG and increasing TG concentration, and even further delayed within treatments of high heat treatments (T_{85}). Similar observation has been made by O'Sullivan *et al.* (2002) and Bönisch *et al.* (2008) in rennet-induced coagulum. Thermal denaturation and aggregation of whey proteins have been extensively studied. Upon heating milk above 65°C, whey proteins are denatured by the unfolding of their polypeptides. The unfolded whey proteins then interact with k -casein which in turn retards the primary phase (lag phase) of rennet coagulation (Dalgleish, 1992; Singh and Waungana, 2001).

Table (2), Yield%, formagraph and rheometer parameters as influenced by heat treatment and TG concentration, as indicated by GLM analysis.

	Yield%	RCT (min)	K20	A30 (mm)	t_c (min)	G'_{30} (Pa)	G'_{60} (Pa)
T_{72} -0U	18.6 ^a ± 0.61	12.23 ^a ± 0.49	6.3 ^a ± 0.9	33.8 ^a ± 0.24	8.52 ^a ± 0.92	82.7 ^a ± 10.4	138.7 ^a ± 15.3
T_{72} -1U	23.8 ^b ± 0.78	13.94 ^b ± 1.14	8.9 ^b ± 1.12	27.5 ^b ± 0.72	10.13 ^b ± 0.57	65.1 ^b ± 11.2	121.4 ^b ± 11.8
T_{72} -2U	27.08 ^c ± 1.15	15.83 ^c ± 1.0	12.9 ^c ± 0.57	20.1 ^c ± 0.58	11.32 ^c ± 0.88	41.3 ^c ± 9.8	99.7 ^c ± 13.3
T_{85} -0U	24.79 ^b ± 1.06	13.01 ^b ± 0.68	8.5 ^b ± 0.84	28.2 ^b ± 0.37	9.22 ^a ± 0.76	58.7 ^b ± 12.3	115.7 ^b ± 11.6
T_{85} -1U	30.81 ^d ± 0.93	17.69 ^d ± 1.0	16.1 ^d ± 0.95	17.2 ^d ± 0.95	12.18 ^c ± 0.59	39.8 ^c ± 10.1	96.3 ^c ± 14.1
T_{85} -2U	37.49 ^e ± 0.57	20.79 ^e ± 0.93	17.1 ^e ± 1.15	12.3 ^e ± 0.80	14.34 ^d ± 0.82	22.1 ^d ± 8.2	75.2 ^d ± 11.7

Values are means of triplicate blocks of three individual milk samples ($n = 9$), ± standard deviation.

Means in the same column bearing a different superscript letter differ significantly ($P \leq 0.01$).

Additionally, the k -casein molecule, which is located at the surface of the casein micelle, possesses four potential glutamine residues that are available as cross-linking sites rendering k -casein susceptible to TG, which consequently elongate the lag phase of rennet-induced coagulation (Sharma *et al.*, 2001; Tolkach and Kulozik, 2005).

Furthermore, the significant increase ($P < 0.01$) of RCT and t_c values of T_{85} -1U and T_{85} -2U treatment clearly indicated that an induced and excessive cross-linked caseins were performed. This is mainly due to the thermal denaturation of whey proteins; particularly β -lactoglobulin, that in turn improve the reactivity of protein cross-linking (Rodriguez-Nogales, 2006). Also, Bönisch *et al.* (2004) has concluded that the severe heat treatment of milk inactivates the TG inhibitor in milk and leads to improve the cross-linking reaction of milk proteins.

As may be seen from Table 2 and the typical graph obtained with rheometer (Fig. 2), the clotting rate (K20) values significantly increased ($P < 0.01$), whereas the slop of each treatment curve was markedly reduced, along with increasing TG concentration and heat treatment. Consistent with this finding, a significant reduction ($P < 0.01$) was obtained in the modulus of elasticity G'_{30min} and G'_{60min} values as increasing TG concentration, which

could be mainly attributed to the introduction of new inter- and intra-molecular covalent bonds in and between milk proteins that resulted in more hydrated texture and less firmness functionality as mentioned earlier. These findings are in conformity with those of Bönisch *et al.* (2008), Özer *et al.* (2012) and Sayadi *et al.* (2013). All these results, however, indicate that not only the primary phase of rennet-induced coagulum may influenced by TG addition as stated by O’Sullivan *et al.* (2002), but in particular the secondary phase of rennet-induced gel and even further the formation of a three dimensional gel structure appeared to be affected significantly by the TG cross-linking (Bönisch *et al.*, 2008; Partanen *et al.*, 2008).

A closer observation of the data shown in Table 2 revealed that although significant increase ($P < 0.01$) in the coagulation time (t_c) was obtained along with increasing TG concentration and heat treatment, the net changed t_c values were not of tangible increase, when their beneficial impact on gel yield is considered. The delay in clotting time started from 1.6 min. in T_{72-1U} treatment up to 5.1 min. in T_{85-2U} treatment, compared to their reference treatments without TG, whereas T_{85-2U} treatment exhibited about double times (101% increase) the curd yield% of T_{72-0U} treatment.

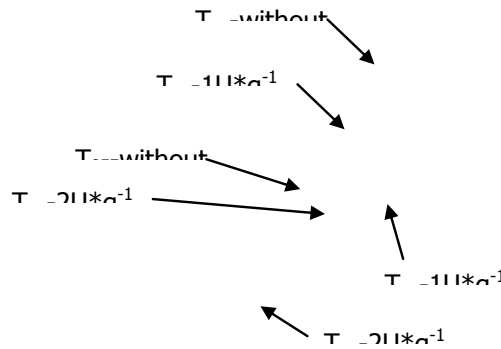


Fig. (2). Influence of heat treatment and TG concentration of low-fat milks on storage modulus G' (Pa) during rennet gel formation. A typical graph obtained with rheometer (Physica-US200).

An attempt is made in Table 3 to explore the correlation between formagraph and rheometer parameters. A significant and negative correlations ($-0.97, P < 0.001$) were obtained between curd firmness (A30) and the clotting time (RCT) as well as curd firming rate (K20) reflecting the impact of TG and excessive heat treatment on extending the primary phase (lag phase) and the secondary phase of rennet coagulation which consequently lead to a markedly reduced curd firmness as discussed earlier. A similar

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trend of correlation ($-0.957, P < 0.001$) obtained between the onset of gelation (t_c) and the gel strength after 60 min reaction time (G'_{60min}).

by another viewpoint, a highly significant ($P < 0.001$) and strong correlation (more than 0.910) among all formagraph and rheometer parameters, which was expected as a similar trend of the rheological properties of milk gels was obtained by the formagraph and rheometer measurements (Table 2). Nevertheless, the significant differentiation pronounced within formagraph parameters; represented in more different superscript letter among low-fat milk gel treatments compared to the outcomes of rheometer variables, might in turn reflect the higher suitability of this technique to define the physical properties of milk coagulation progress. However, it is worthwhile to state that rheometer possess a very wider range of techniques and can conduct more analysis to explore the physical properties of milk that are not capable to be performed by the formagraph.

Table (3). Simple correlation matrix and *P*-value between formagraph and rheometer parameters, as indicated by GLM analysis.

	RCT	K20	A30	Tc	G'30
K20	0.957***				
A30	-0.969***	-0.972***			
Tc	0.989***	0.949***	-0.964***		
G'30	-0.918***	-0.936***	0.971***	-0.938***	
G'60	-0.946***	-0.938***	0.975***	-0.957***	0.984***

*** Correlation is significant at 0.001 level.

Microstructure: The CLSM technique was used to differentiate between the structural components of the milk gels by the combination of specific stains. Moreover, the CLSM was recommended as a very convenient analytical method for gels with high water content as recommended by Myllärinen *et al.* (2007) and Lopez *et al.* (2007). The effects of heat treatment as well as TG implementation on the microstructure of rennet-induced low-fat milk gels are shown in Fig. 3. The protein matrix (red area) formed a continuous phase permeated by an amorphous system of voids filled with serum (black area), whereas the milk fat globules appear as discrete green spots uniformly dispersed and/or embedded throughout the protein matrices.

Confocal micrograph shown in Fig. 3B clearly revealed that the protein network in low-fat milk gel of higher heat treatment (T_{85}) appeared to be made up from rather densely aggregated protein particles with homogeneous systematic network accompanied by small pores. While in Fig. 3A, the lower heat treatment (T_{75}) appeared to have irregularly aggregated protein matrix interrupted by larger pores reflecting its coarser structure. The homogeneous and increased network formation obtained by increasing heat treatment of milk might be attributed to the interference of denatured whey proteins into the gel network (Singh and Creamer, 1992). The denatured whey proteins can associate with the casein micelles, involving *k*-casein via thiol-disulphide interchange and results in increased bridging within the gel, giving threadlike appendages and branched kind of network (Lucey *et al.*, 1998; Considine *et al.*, 2007).

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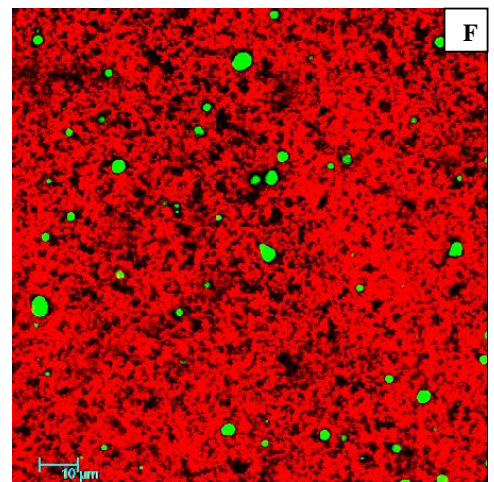
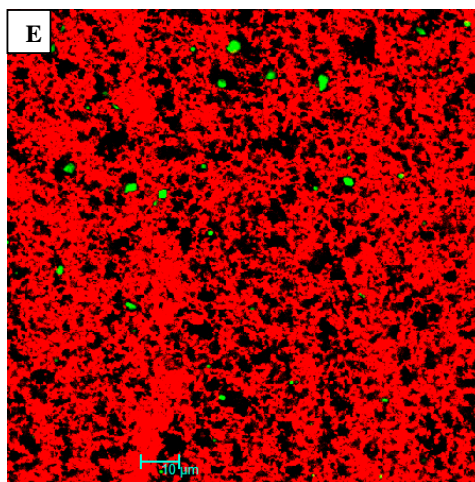
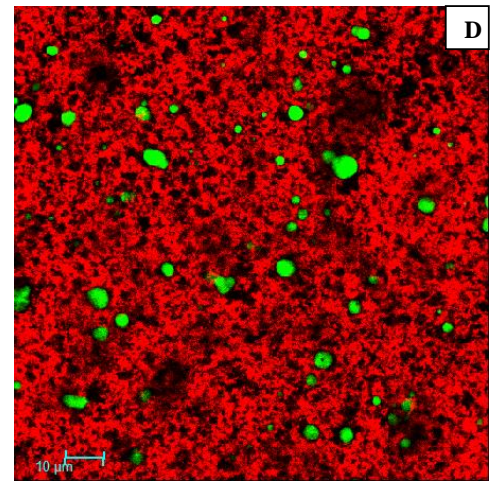
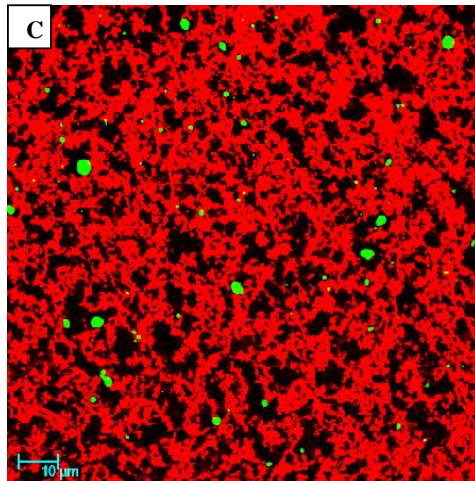
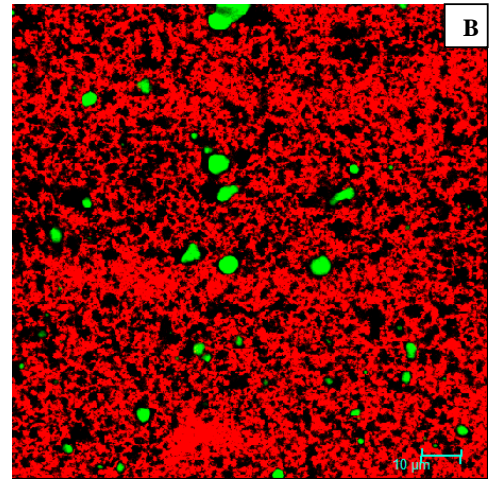
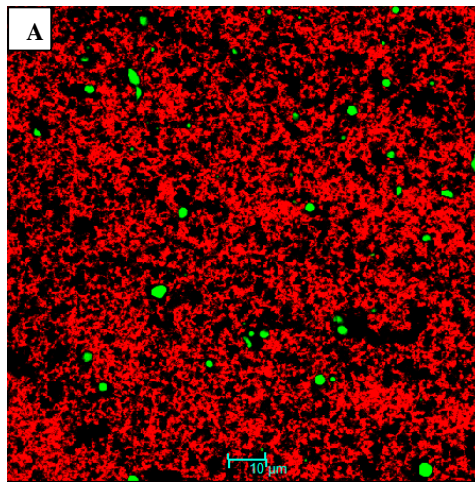
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Additionally, addition of TG to low-fat milk treatments (Figs. 3C-3F) promoted regularly aggregated protein matrices and interconnectivity of the network accompanied by homogeneous systematic pores that obviously were much finer compared to that of control treatments without TG (Figs. 3A and 3B). It is worthwhile to note that the impact of TG on microstructure characteristic was more pronounced as TG concentration increased, and was even further intense by increasing heat treatment as illustrated in confocal images for T₇₂-2U and T₈₅-2U treatments. Obviously, increasing TG concentration results in much smooth protein network consisting of finer-meshed aggregates accompanied by rather small and evenly distributed pores. These changes in the protein matrix could be mainly attributed to the capability of TG to form both inter- and intra-molecular isopeptide protein bonds (Kuraishi *et al.*, 2001), in addition to improving the reactivity of protein cross-linking resulted from high heat treatment of low-fat milks (T₈₅), as discussed earlier. These observations are in accordance to those reported by Kruif *et al.* (2002), Myllärinen *et al.* (2007) and Partanen *et al.* (2008).

The different sizes of fat observed in confocal images of Fig. 3 could be explained by the attempt of Lopez *et al.* (2007) using CLSM technique to investigate the fat distribution within protein matrix, and have concluded that a three phases of fat dispersion could be obtained within protein matrix: as individual small fat globules embedded in the protein matrix, as aggregates of fat globules, and finally as larger more irregularly shaped fat globules. The authors further noted that the last shape of fat globules may play the role of breakers of the casein network, as the pores of casein network smaller than this fat phase.

Heat treatment 72°C/15sec. [A, C and E]

Heat treatment 85°C/5min. [B, D and F]



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Fig. (3). CLSM micrographs (63x) of low-fat milk gel treatments; A) T₇₂-0U, B) T₈₅-0U, C) T₇₂-1U, D) T₈₅-1U, E) T₇₂-2U, F) T₈₅-2U.

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A closer observation of the microstructure details in Fig. 3 revealed that the milk fat globules were more pronounced and more uniformly scattered throughout the protein matrices in the T₈₅ low-fat milk gel treatments (Figs. 3B, 3D and 3F) compared to the structures of T₇₂ low-fat milk gel treatments (Figs. 3A, 3C and 3E), despite the fact that a uniform fat content was achieved in all low-fat milk gel treatments. This finding could be mainly correlated to the promoted protein aggregates of fine network strands and small pores obtained in the T₈₅ treatments, which tended to include discrete fat globules differing in size within the protein matrix in different manner.

These findings together with the more hydrated texture obtained in T₈₅ treatments as indicated by its curd yield after centrifugation data (Table 2), may contribute to their softer texture as indicated by the rheological indices (Table 2 and Fig. 2). In this context, Lucey *et al.* (2001) have concluded that gels with large pores would have a lower water holding capacity as water would find it easier to move to the gel surface. Besides, Schorsch *et al.* (2000) concluded that addition of TG to milk gels presented a finer network with thinner particle strands and smaller pores, leading to less milk gel syneresis.

CONCLUSION

Considerable progress has been made toward understanding how TG protein cross-linking and heat treatment of milk affect rheological and microstructure properties of rennet-induced low fat milk gels. This study demonstrated that low-fat milk gels of interesting features in term of strength, kinetics of gelation (time and rate) and yield could be formed as a function of the experimental factors, either individually or by their interactions. The results show that the impact of TG on the rennet coagulation properties, appears more intense in the secondary phase measurable by intensive reduction in curd firmness (A_{30}), declined rigidity rate of casein network (G'_{30min}) and markedly reduction in gel strength (G'_{60min}), than in the resulting prolongation of the coagulation times (RCT and t_c). This effect is more pronounced for higher TG concentrations and higher heat treatment of milk. Likely, the thermal denaturalization of whey proteins as well as the rearrangement of casein micellar structure improves the reactivity of protein substrate towards protein cross-linking.

Qualitatively, the rheological results obtained by formagraph technique were strongly correlated with those obtained by rheometer measurements. Although, the rheometer is well-known by its physical highly perception sensitivity, formagraph has exhibited much ability to differentiate the milk clotting variables among the present experimental factors.

The TG cross-linked gels exhibit as observed by CLSM a more continuous, dense and smooth matrix, accompanied by homogeneous systematic and much smaller pores, compared to their control gels without TG. CLSM imaging of low-fat milk gels texture provides a qualitative approach to follow-up the changes observed in rheological indices.

In conclusion, heat treatment of milk combined with TG manipulations may offer manifold possibilities and a promising option to direct the industrial processes to the enhancement and optimization of low-fat dairy varieties, with particular respect to soft cheeses, semi-hard cheeses and novel functional dairy products.

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المخلص العربي

تأثير الترابط التعمادي للإنزيمي للبروتين علي الخواص الريولوجية والتركيب البنائي الدقيق لخبثرة التجبن الانزيمي للبن البقري منخفض الدهن

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كان لإنزيم الترانسجلوتاميناز الميكروبي في الأونه الأخيرة نصيب كبير من البحث و الدراسة في مجال الألبان ومنتجاتها ، لما له من قدرة عالية على تعديل الخصائص الفيزيائية و التركيبية للمنتجات اللبنية. و لذلك تناولت هذه الدراسة تأثير تركيز إنزيم الترانسجلوتاميناز المضاف للبن وكذا تأثير معاملة اللين حرارياً علي الخواص الفيزيائية و التركيب البنائي الدقيق لخبثرة اللين البقري منخفض الدسم (١,١%دهن) الناتجة من التجبن الإنزيمي. و قد تمت دراسة الصفات الريولوجية للخبثرة أثناء فترة التجبن الإنزيمي بواسطة جهازى الفورماجراف (formagraph) و الريومتر (dynamic rheometer) و تم استخدام التحليل الاحصائى لايضاح مدى الارتباط و معنويته بين القياسات الفيزيائية من كلا من الجهازين. و قد دللت نتائج الدراسة على قوة ظهور تأثير إنزيم الترانسجلوتاميناز على خصائص التخثر الإنزيمي للبن في الطور الثانوي الذي يستدل عليه بالزيادة الواضحة في تصافي الخبثرة الناتجة عن الطرد المركزي وكذلك التناقص الملحوظ في قوة ثبات الخبثرة و إنحدار معدل الصلابة لشبكة الكازين مقارنة بتأثير الإنزيم في الطور الابتدائي للتجبن و المتمثل فقط في إطالة زمن التجبن. و تلك التأثيرات كانت أكثر وضوحاً مع التركيزات العالية من إنزيم الترانسجلوتاميناز و المعاملات الحرارية العالية للبن. كما أدت معاملة اللين بإنزيم الترانسجلوتاميناز الى تحسين تجانس التركيب الدقيق للخبثرة ، و هو ما دلل عليه صور الميكروسكوب الماسح بالليزر (Confocal Laser scanning microscope) حيث أظهر التركيب البنائي الدقيق للخبثرة المتكونة من اللين المعامل بالترانسجلوتاميناز الشبكة البروتينية بصورة أكثر اندماجاً و تجانساً في التركيب و البناء و ايضاً اقل في حجم المسام الموزعة بتجانس مقارنة بخبثرة اللين الغير معمل ، وازدادت تلك الخصائص وضوحاً مع التركيزات العالية من الترانسجلوتاميناز و ارتفاع المعاملة الحرارية للبن. كما لوحظ ارتباطاً معنوياً قوياً بين نتائج dynamic rheometer و نتائج formagraph.

ووصفة عامة يمكن الاستنتاج ان استعمال انزيم الترانسجلوتاميناز و خاصة مع المعاملات الحرارية العالية للبن قد أدى الي تحسين الصفات الوظيفية و التركيبية بصورة واضحة للخبثرة الإنزيمية للبن البقري منخفض الدسم مما يسمح باستخدامه في تطبيقات متعددة و خاصة التي تتعلق بتحسين خصائص الجودة للجبين منخفض الدهن.

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